THE QUANTITATIVE DETERMINATION OF THE SPECTRAL DISTRIBUTION OF PHOTOTACTIC SENSITIVITY IN THE PURPLE BACTERIUM *RHODOSPIRILLUM RUBRUM*

by

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INTRODUCTION

The reactions of freely moving organisms to light are termed phototactic movements or more briefly phototaxis. Phototaxis is described in motile animals as well as in motile plants such as green algae, flagellates and purple bacteria. Photic orientation in one of this lower plants, the purple bacterium *Rhodospirillum rubrum*, was subject of the present investigation.

We owe most of our knowledge of phototaxis in purple bacteria to the brilliant investigations of ENGELMANN[1,2,3]. In order to examine the behaviour of these light-sensitive organisms, this investigator exposed them to a micro-spectrum which was projected into the slide by means of a micro-spectral apparatus. With the aid of this apparatus ENGELMANN found that the bacterium investigated (*Bacterium photometricum*) collected in bands in the infra-red region of the micro-spectrum between 9000–8000 A, in a somewhat less distinct band in the yellow at about 5900 A and in a still less distinct band in the green between 5500–5200 A. Afterwards, BUDER[4], repeating this in experiments with other motile purple bacteria, succeeded in splitting up the region of aggregation in the green into three very weak, it is true, but nevertheless rather distinct regions at about 5300, 4900 and 4700 A.

Accumulation of phototactic organisms in a spectrum which partially illuminates the microscopic field is due to the organisms' property of executing a so-called "shock reaction" (avoiding reaction) on the sudden reduction in the light intensity which the organisms undergo in entering the dark zone. As a result of this phototactic reaction, the direction of movement is reversed, which prevents the organisms from crossing the boundary from light to dark. Since, conversely, the organisms are not prevented in their random movements from entering the illuminated part out of the dark, a light zone acts like a trap. Consequently these bacteria accumulate at the parts of relatively highest light intensity.

The accumulation of purple bacteria in the micro-spectrum in certain spectral bands makes it highly probable that the zones of different wave-lengths are perceived by the bacteria as spots of different intensity. So it may not be due to colour distinction. Apparently, different wave-lengths are to a smaller or larger extent absorbed by the

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bacteria, those being absorbed to a large extent being situated in the spectrum at the zones of an accumulation.

ENGELMANN's and BUDER's experiments with phototactic organisms in a microspectrum give only a rough estimate of the spectral distribution of phototactic sensitivity, since they could not eliminate the influence of energy distribution in their spectrum. Moreover, the experiments of ENGELMANN and of BUDER were arranged in such a way that aggregations only occurred in the top regions of the absorption bands of the pigments involved in phototaxis. In this way an impression of the height of these maxima or the magnitude of the phototactic action as a function of wave-length can not be obtained.

Principle of the bacteriophotometer

Since the experiments of ENGELMANN and of BUDER are of a *qualitative* nature, it is impossible to derive the *action spectrum* of phototaxis from their data. For the purpose of obtaining such an action spectrum, we employed the *quantitative* principle of a compensation method.

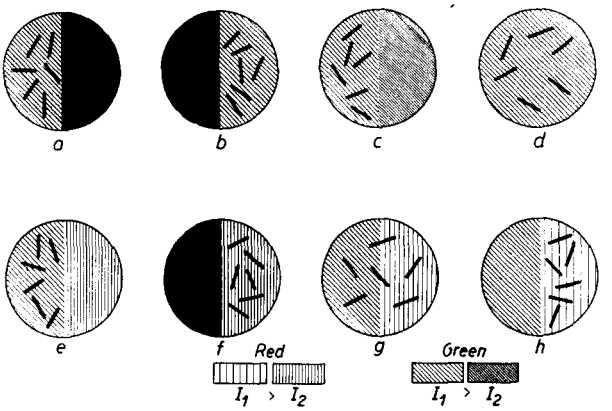

Fig. 1. Principle of the way in which the bacteriophotometer acts. Phototactic response of the bacteria to eight light conditions of the microscopical double field. Intensity indicated by line distances.

On a slide under the microscope two small light fields were projected adjacent to each other with a sharp boundary. The illumination of one of these fields could be varied in intensity as well as in wave-length, comparable with the situation in a photometer according to LUMMER-BRODHUN, in which one of the fields is also variable in intensity. For this reason the set-up in which the principle of compensation was used was named "the bacteriophotometer"; light-sensitive bacteria (*Rhodospirillum rubrum* Strain 4) serving as an "indicator" in order to establish in which of the fields the light intensity is highest.

The following description may explain in which way the bacteriophotometer acts. Fig. 1 shows eight double fields, indicated as *a*, *b*, *c*, *d*, *e*, *f*, *g*, and *h*. Only (monochromatic) green light is used in the cases given in *a*, *b*, *c*, and *d*. In *a* and also in *b* only one field is illuminated. Consequently aggregation of spirilla occurs only in this field. When both fields are illuminated by the same light source, the right field having a higher intensity than the left one (*cf. c*), the bacteria are collected in the former. If one spirillum moves into the direction of the boundary between both fields, the direction of motion

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is reversed as soon as it has entered the field of relative lower intensity, in accordance with the rule that a shock reaction is released upon a sudden reduction of the light intensity (see above). Only in the case that the intensity of the fields is equal (*cf. d*) are the bacteria distributed homogeneously over both fields. In the set of figures *e*, *f*, *g* and *h* the phototactic action of red light is compared with green. When the intensity of the light in the green and red field are the same in a physical sense, accumulation of spirilla occurs only in the green one (*cf. e*). By blacking out the green field (*cf. f*), aggregation takes place in the red. Apparently, green light possesses a higher activity in phototaxis than red. By increasing the intensity of the red field, it is possible to obtain again a homogeneous distribution of the bacteria over both fields, in which case the bacteriophotometer is "adjusted to equivalence" (*cf. g*). Increasing still more the intensity of the red field leads to an accumulation in the red (*cf. h*).

In the case of adjustment to equivalence on passage of the boundary no shock reactions of the bacteria are observed. If in such a case the intensity of the red field —expressed in quanta/cm² sec*—was, *e.g.*, 2 to 3 times that of the green one, this means that the phototactic efficiency of red light is 2 to 3 times less with respect to green. Consequently the *phototactic* efficiency of the red with respect to the green is ½ to ⅓, *i.e.* the ratio $\frac{I_{\text{green}}}{I_{\text{red}}}$; I_{green} and I_{red} being the intensity in quanta/cm² sec of the green and red field respectively. Substituting the red light by light of other wave-lengths, and again adjusting the bacteriophotometer to equivalence with the standard green field, the ratio $\frac{I_{\text{green}}}{I_{\lambda}} = W_{\lambda}$ can be determined for each wave-length. Plotting the values of W, determined in this way, in a diagram versus wave-length, a curve is obtained which, as will appear presently (see below), represents the action spectrum (*cf.* Fig. 5).

Demonstration of the identity of the action spectrum as defined above and the absorption spectrum of the light acceptor active in the phototactical reaction

The experiments of ENGELMANN and of BUDER regarding the accumulation of purple bacteria in certain spectral bands demonstrate a relation between the spectral distribution of phototactic sensitivity and the absorption spectrum of the cell pigments. This is evident with a view to GROTTHUS-DRAPER's rule, which assumes only absorbed light to be effective. From an extension of EINSTEIN's law, for the first time formulated and applied to photochemistry by WARBURG[5], it must be concluded that only the *number* of quanta absorbed, and not their *energy content* is essential. Assuming EINSTEIN-WARBURG's law to be valid in our case also (FRENCH[6]) the photobiological effect W'_{λ} of radiation is a function of $I_0 - I$, the number of quanta/cm² sec absorbed by a bacterium. So the following equation holds:

$$W'_{\lambda} = \mathrm{f}(I_0 - I), \tag{1}$$

where W'_{λ} is the photobiological effect of the radiation with the wave-length λ with an incident intensity I_0 (expressed in quanta/cm² sec) and I the intensity of radiation (also expressed in quanta/cm² sec) after having passed a bacterium.

* Assuming EINSTEIN-WARBURG's law[5] to be valid for the photochemical process initiating phototaxis, the activity of the light is proportional to the number of quanta absorbed by the spirilla. Hence, the intensity of the light must be expressed in quanta/cm² sec.

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Taking into account that the distribution of pigment in a bacterial cell is possibly not homogeneous, and considering the fact that the light path of various beams inside the bacterium is of different lengths, LAMBERT-BEER's law can only be applied for an infinitely thin monochromatic beam of light (cross section dO) in the following form:

$$I\mathrm{d}O = I_0\mathrm{d}O.\mathrm{e}^{-a_\lambda \int_0^l c_x\,dx}, \tag{2}$$

where IdO (expressed in quanta/sec) is the intensity of the light beam after absorption over the distance of the absorbing layer l, I_0dO (also expressed in quanta/sec) the initial intensity of the beam, e the basis of natural logarithms, a a specific factor that only depends on the wave-length and is called the absorption coefficient, and c_x the concentration of the pigment along the beam.

The amount of the light beam absorbed is:

$$I_0\mathrm{d}O - I\mathrm{d}O = I_0\mathrm{d}O - I_0\mathrm{d}O.\mathrm{e}^{-A} = I_0\mathrm{d}O\,\{1 - \mathrm{e}^{-A}\}, \tag{3}$$

when $a_\lambda \int_0^l c_x dx$ is called A.

Since the decrease in light intensity in a single bacterium is negligibly small, each spirillum being entirely colourless under the microscope, it may be assumed that practically no absorption takes place. This indicates that the factor $a_\lambda \int_0^l c_x dx = A$ in formula (2) is very small ($A \ll 1$). Therefore, we may simplify to:

$$\mathrm{e}^{-A} = 1 - A. \tag{4}$$

$$\text{or} \quad 1 - \mathrm{e}^{-A} = A. \tag{5}$$

It follows from (3) and (5) that:

$$I_0\mathrm{d}O - I\mathrm{d}O = I_0\mathrm{d}O\,A. \tag{6}$$

For the total amount of light absorbed by the bacterium we have to integrate over the total cross-section s of the light beam:

$$I_0 - I = \int_s \{I_0\mathrm{d}O - I\mathrm{d}O\} = \int_s I_0\mathrm{d}O.a_\lambda \int_0^l c_x dx = I_0.a_\lambda \int_s \mathrm{d}O \int_0^l c_x dx. \tag{7}$$

From (1) and (8) it follows that:

$$W'_\lambda = \mathrm{f}\left\{I_0.\alpha_\lambda \int_s \mathrm{d}O \int_0 c_x dx\right\} \tag{8}$$

Equation (8) shows that the photobiological action of a light field is a function of the intensity in quanta/cm² sec, and the absorption coefficient of the pigment involved in phototaxis. If the bacteriophotometer is adjusted to equivalence *e.g.* for red and green light, we have for both fields:

$$W'_{\text{red}} = W'_{\text{green}}.$$

or:

$$\mathrm{f}\left\{I_{\text{red}} \cdot \alpha_{\text{red}} \int_s \mathrm{d}O \int_0^l c_x dx = \mathrm{f}\left\{I_{\text{green}} \cdot \alpha_{\text{green}} \int_s \mathrm{d}O \int_0^l c_x dx. \right.\right. \tag{9}$$

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As f is shown to be a univalent function (*cf.* MANTEN[7]), we may simplify to:

$$I_{\text{red}} \cdot a_{\text{red}} \int_s \mathrm{d}O \int_o^l c_x dx = I_{\text{green}} \cdot a_{\text{green}} \int_s \mathrm{d}O \int_o^l c_x dx. \tag{10}$$

The factor $\int_s \mathrm{d}O \int_o^l c_x dx$ is independent of the wave-length in one single spirillum.

As the bacteriophotometer is a single-cell method we are allowed to cancel this factor:

$$I_{\text{red}} \cdot a_{\text{red}} = I_{\text{green}} \cdot a_{\text{green}}. \tag{11}$$

We have defined the phototactic action as $W = \frac{I_{\text{green}}}{I_{\text{red}}}$, and it follows from (11):

$$W = \frac{I_{\text{green}}}{I_{\text{red}}} = \frac{a_{\text{red}}}{a_{\text{green}}}. \tag{12}$$

With respect to the action of other wave-lengths, the general equation holds:

$$W_\lambda = \frac{I_c}{I_\lambda} = \frac{a_\lambda}{a_c}. \tag{13}$$

in which I_c and a_c refer to the green standard field and thus are constants.

We see from (13) that the light intensities required for an adjustment to equivalence are inversely proportional to the absorption coefficients of the pigment complex in which phototactically active light is absorbed. Therefore, the action spectrum of phototaxis as earlier defined may be considered to be identical with the absorption spectrum of the light acceptor active in phototaxis.

Experimental set-up of the bacteriophotometer

The action spectrum of phototaxis was measured with the help of the arrangement shown in the scheme of Fig. 2.

We intended to obtain two homogeneously illuminated fields under the microscope. As the fields had to be illuminated homogeneously (variation limit 10%), the size of each of the fields was limited, each of the rectangularly shaped fields being only 0.2 mm long and 0.15 mm wide.

Both fields at a'–b' (Fig. 2) are focussed in the plane of the slide by the condenser C, and correspond to the field a–b in the double prism P. The part a–c of the diagonal plane between the right-angled prisms in P reflects the light, whereas the part c–b transmits it. The prisms are partially cemented by a very thin layer of Canada balsam. In this way it is possible to obtain a very sharp boundary between both fields. This is necessary in order to prevent the bacteria from perceiving the

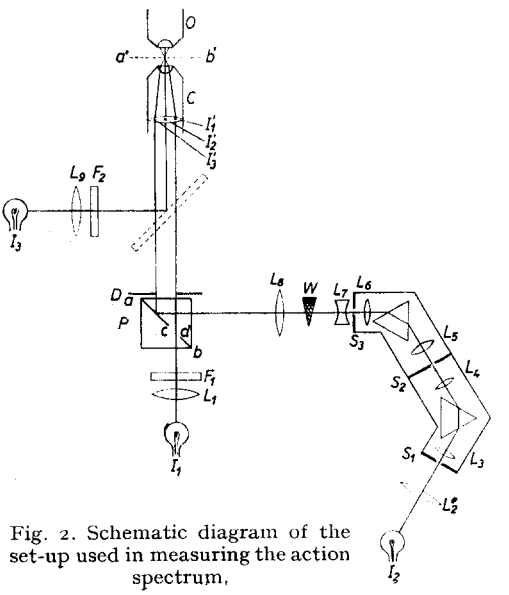

Fig. 2. Schematic diagram of the set-up used in measuring the action spectrum.

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boundary as a range of different intensity which might produce shock reactions, even when the illumination of both fields is equal. The light source I_1, a motor-car lamp (Philips 6 V; 6 A), illuminates the right field c–d. The source of light I_2 is a so-called projector lamp (Philips 6 V; 7 A, with mirror) the upright incandescent spiral of which is focussed upon the slit S_1 by the lens L_2, illuminating the left field a–c via the monochromator and the weakener W. With a view to the required constancy of the fields at a–b and a'–b' both lamps are fed by the same large storage battery.

The monochromator according to VAN CITTERT[8] is adjusted in such a way that the loss of light is as small as possible. By adjusting the middle slit S_2 the monochromator can be made to transmit light of every desired wave-length. In order to get a homogeneously illuminated field in the double prism P at a–b, and on the slide at a'–b', the concave lens L_7 is mounted immediately in front of the end slit S_3, so that the plane of the homogeneously illuminated lens L_6 is focussed in the double prism upon the plane through a–b. The light beam at the same time passes the lens L_8 and the adjustable logarithmic weakener W. The image of the slit S_3 is projected by the lens L_8 via the reflecting part of P, in the plane of the large lens of the condenser C. As in the case in which the light comes from the lens L_1, which is placed as close as possible to the double prism P, the light from the slit S_3 is thus also focussed in a homogeneously illuminated field at a'–b'.

Finally, the light source I_3, for which a 12 Volt motor-car lamp was used, illuminates the whole microscopic field with a weak phototactically-active yellow-green light. This illumination was used in order to be able to detect the bacteria also, when working with infra-red radiation. A beam from this source passes the lens L_9, then the set of filters F_2, being identical with F_1 (Schott filters VG_1 mm + 2 × OG_2 mm + 1 cm 16% $CuSO_4$ solution, the transmission-spectrum of this set shows one band at 5600 A), and is reflected by a glass plate which acts as a semi-transparent mirror. By this system a strongly weakened image of the incandescent spiral is focussed on the condenser C. The spiral of I_1 is focussed in such a way that its image crosses that of the slit S_3 at right angles. The image of the incandescent spiral of I_3 is projected over the intersection of the images of I_1 and S_3. This intersection is projected exactly in the opening of the diaphragm of the condenser C. The aperture of the diaphragm is about 2.5 mm. A wider aperture has an unfavourable effect, probably due to reflections in the slide. This sets an upper limit to the intensity of the fields.

As it was necessary to maintain a sharp boundary between the fields, even when working with strongly different wave-lengths, the normal condenser with its great chromatic aberration was replaced by an achromatic Leitz No. 4 objective of about the same focus.

The procedure of the measurement was as follows. The yellow-green light transmitted by the system of filters F', illuminating one of the two fields, was used as a constant light, with which the light from I_2, adjustable in intensity by the logarithmic weakener W and in wave-length by S_2, was compared. Then, for each wave-length the position of the weakener W was noted, at which a shock reaction could just be perceived in some spirilla, which were evidently more sensitive to contrasts than the others, when they crossed the boundary. In this way for each wave-length two positions of the weakener were found, corresponding with two light intensities. Between these positions no shock reaction of the bacteria occurred when they passed the boundary.

The measurement of the actual light intensities was carried out with a micro-

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vacuum-thermo-element which was especially constructed for measurements under the microscope, and was connected with a very sensitive Zernike galvanometer.

The receiver of the thermo-couple according to MOLL (*cf.* 9) consisted of a small band of rolled out "thermo-plate". of only 50 μ width and 1 μ thickness. This small band was stretched between the copper leads of a lamp base, blackened by means of a thin layer of soot and finally sealed into a T-shaped glass tube which was supplied with two plan-parallel windows (Fig. 3). The thermo-element was evacuated by means of a double-stage mercury diffusion pomp, during which the element was heated at 150° C. Finally, the pressure of the mercury vapour was diminished during pumping by a treatment with liquid air. After this procedure the sensitivity of the element had increased about 90-fold. The glass-covered system (Fig. 3) was enclosed in a case of brass with two round openings for the windows. During the intensity measurement the beam-shaped brass case was clamped upon the object-table of a Zeiss microscope, provided with a mechanical stage. Then at a small magnification the most sensitive spot of the small receiver, the welding point, was brought into a field close to the boundary. As for an adequate determination of the intensity it was absolutely necessary to irradiate always exactly the same part of the small thermo-band, the fields had been given a rectangular form, and care was always taken to illuminate exactly the same part of the small thermo-band. The fields were about 0.2 mm long and 0.15 mm wide. As the width of the blackened small thermo-band was about 0.07 mm, this could thus be amply comprised twice in the width of each field.

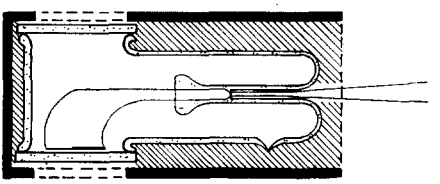

Fig. 3. Cross-section of the micro vacuum-thermo-element.

The most sensitive spot of the small thermo-band could easily be traced by projecting an image of a slit of about 0.1 mm width in the field of the microscope and moving the small band across in a perpendicular direction. By reading the deflection of the galvanometer for each position of one of the adjusting buttons of the mechanical stage, the distribution of the sensitivity was found as shown in Fig. 4. The sharp maximum in this figure is striking. Because of this, it was desirable to mark the most sensitive spot under the microscope, so as to be able to find it again. This was done by engraving a small circle on the front window by means of a marking instrument.

The absolute calibration of the thermo-element was carried out in the following way. A little diaphragm was mounted on the window above the sensitive part of the thermo-element. Its opening was such that exactly the same part of the surface of the small thermo-band was irradiated, as was the case when measuring the intensity of the small fields under the microscope. Then

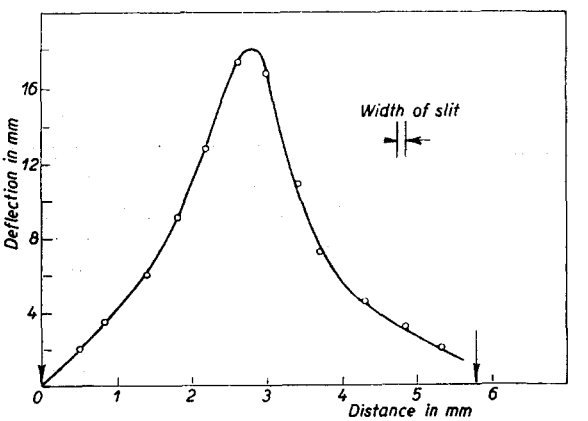

Fig. 4. Distribution of the sensitivity over the thermo-band of the micro vacuum-thermo-element.

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it was calibrated, using white light from a 12 V motor-car lamp, which was connected to a storage battery and placed at a distance of 20 cm from the element. The deflection of the Zernike galvanometer obtained in this way was compared with the deflection obtained with an absolutely standardized thermopile.

It must be remarked that the sensitivity of the absolutely calibrated thermo-element does not depend on the wave-length if the intensity of the light is expressed in ergs. Since it was necessary to correlate the phototactic action with the light intensity expressed in quanta/cm² sec, the intensities read in ergs/cm² sec were calculated into quanta/cm² sec.

The action spectrum

The action spectrum was obtained as follows. Both limit values of the intensity, between which the spirilla did not show any shock reaction on passage of the boundary, were determined for each wave-length. This was done by adjusting to equivalence with

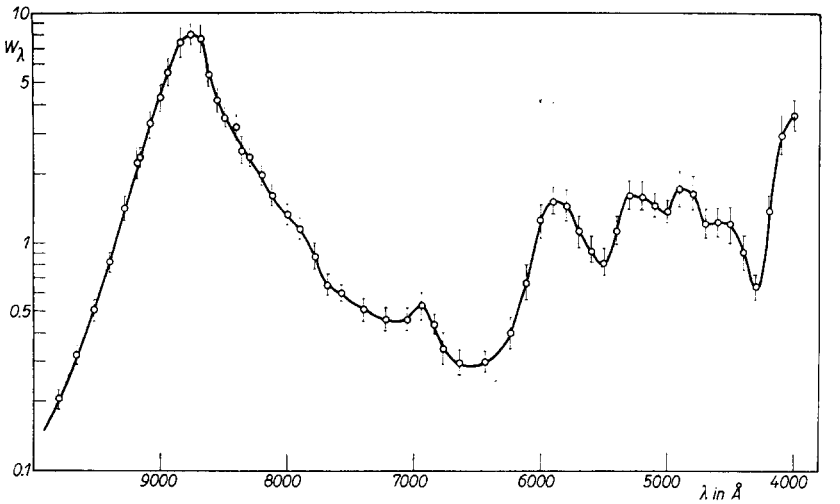

Fig. 5. Phototaxis action spectrum.

the aid of increasing as well as decreasing intensities of the variable field. These intensities, expressed in quanta/cm² sec, were divided by the intensity in quanta/cm² sec of the green standard field, and the log-reciprocal values of the proportions were plotted against the wave-length. By drawing a smooth curve through the middles of the intensity margins the action spectrum shown in Fig. 5 was obtained.

The action spectrum as measured with the bacteriophotometer proved to be independent of the absolute intensity of the green standard field below a value of about 10,000–20,000 ergs/cm² sec. If the intensity of the green field *e.g.* is decreased to one-third of the original intensity, then, in order to obtain equivalence, the intensity of the adjustable field must also be diminished to one-third of the original value. Figures indicating the action of infra-red radiation of 8800 A with respect to green of 5600 A, determined in parallel experiments, are given in Table I.

From this table it may become evident that the phototactic action of infra-red with respect to green appears to be constant, without reference to fluctuations due to

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TABLE I

Int. in ergs/cm² sec of the green field	*Intensity ratio infra-red: green. (Int. expressed in ergs/cm² sec)*	*Action infra-red: green. (Int. expressed in quanta/cm² sec)*
9200	14.9	9.5
6700	13.7	8.7
4000	14.0	8.9
2600	15.3	9.7
1140	14.4	9.1

experimental sources of error*. This demonstrates the curve in Fig. 5 not to be influenced by the conditions of the experiments. Consequently, we may consider the curve to represent merely a characteristic for the kind of bacteria investigated, and we are thus allowed to designate it as the action spectrum of phototaxis. As pointed out above this does not hold well for the results obtained by ENGELMANN[1,2,3] and by BUDER[4] which were influenced by the experimental conditions (*e.g.* distribution of energy in the spectrum used) to a large extent. It therefore seems better to speak of relative spectral distribution of phototactic sensitivity in their case.

Final remarks

The action spectrum of Fig. 5 shows that the regions of maximum phototactic action are located at about 8800, 6900, 5900, 5250, 4900 and 4000 A; this being in good agreement with the data given by ENGELMANN[1,2,3] and by BUDER[4]. The small maximum at 6900 A has not been recorded as yet.

The mathematical derivation given above showed the action spectrum to be the absorption spectrum of the light-sensitive substance in which the light acting in phototaxis is absorbed. Subsequent experimentation involving the isolation of the complete pigment system and separation of its components (*cf.* MANTEN[7,10]) showed that the absorption spectrum of the total pigment system is not in full agreement with the action spectrum. This deviation appears to be due to the fact that the light absorbed by spirilloxanthin—which carotenoid occurs in abundance in the spirilla—is not, or only to a much lesser degree, phototactically active (*cf.* Fig. 6). In contrast hereto carotenoids less abundant than spirilloxanthin, and similar to rhodopin or rhodopurpurin—together with bacteriochlorophyll—were found to be involved in the absorption of light active in phototaxis.

Subsequent experiments[7,10] further elucidated that in purple bacteria a simple relation exists between phototaxis and photosynthesis. It was possible to connect the decrease in contrast-sensitivity of the bacteria at high light intensities with the attainment of light saturation in photosynthesis—*cf.* also 11. From this, and from the fact that bacteriochlorophyll is involved in phototaxis, the conclusion was drawn that a phototactic reaction (shock reaction) is released upon a sudden change in the rate of photosynthesis. This implies that the action spectrum of phototaxis in *Rhodospirillum rubrum* is the same as the action spectrum of photosynthesis (*cf.* 12). Consequently,

* The magnitude of the error of measurement is computed herefrom as follows. The average of the figure in the right column is 9.2. The sum of the deviations of this average is 1.7; hence the mean deviation is 0.34. This corresponds to an error of measurement of only 3.7 %, which demonstrates the high accuracy of the bacteriophotometer.

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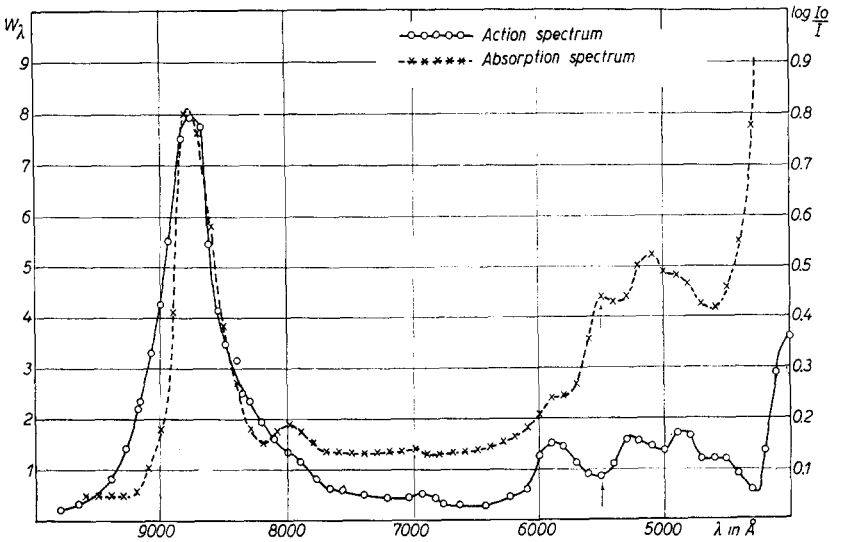

Fig. 6. Absorption spectrum (x) and phototaxis action spectrum (o). Arrows indicate the location of the main absorption peak of spirilloxanthin.

also in purple bacteria light absorbed in certain carotenoids is assumed to be active in photosynthesis. In this respect we may also refer to DUYSENS[13]).

The authors thank Dr J. B. THOMAS for his valuable discussions.

SUMMARY

By using a compensation method, the action spectrum (spectral distribution of stimulating efficiency in a quantitative measure) of phototaxis in the purple bacterium *Rhodospirillum rubrum* (*Esmarch*) *Molisch* Strain 4 was determined. Two differently coloured adjacent small light fields were projected in a slide chamber containing a (dilute) suspension of moving spirilla. One of the fields acted as a standard, whereas in the other one the intensity as well as the wave-length of the light could be varied. The principle of the determination of the spectral sensitivity in the spirilla was based upon the adjustment to equivalence of the two fields for different wave-lengths. Shock reactions of the spirilla at the boundary between the fields were used as an "indicator" as to how far the fields were adjusted.

A mathematical derivation was given. It showed that the action spectrum of phototaxis is identical with the absorption spectrum of the light acceptor active in the phototactical reaction.

The action spectrum shows maxima at about 8800, 6900, 5900, 5250, 4900, 4600, and at about 4000 A.

RÉSUMÉ

Nous avons déterminé le spectre d'action de phototaxie (expression quantitative de la distribution spectrale d'efficacité d'irritation) chez *Rhodospirillum rubrum* (*Esmarch*) *Molisch* S 4 au moyen d'une méthode de compensation. Deux champs lumineux adjacents de couleur différente étaient projetés dans une suspension diluée de bactéries en mouvement sur une lame porte-objet. L'un des champs fonctionnait comme étalon, tandis que, dans l'autre, il était possible de faire varier l'intensité et la longueur d'onde. Le principe de détermination de la sensibilité spectrale des bactéries était basé sur la mise en équivalence des deux champs à des longueurs d'ondes différentes. Les réactions d'évitement des bactéries nous indiquaient jusqu'à quel point les champs lumineux étaient ajustés.

Une dérivation mathématique à été présentée. Elle démontre que le spectre d'action de phototaxie est identique au spectre d'absorption de la substance, par laquelle la lumière active en phototaxie est absorbée.

Le spectre d'action montre des maxima à 8800, 6900, 5900, 5250, 4900, 4600 et 4000 A environ.

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ZUSAMMENFASSUNG

Das Wirkungsspektrum (quantitativer Ausdruck der spektralen Verteilung der Wirksamkeit) der Phototaxis von *Rhodospirillum rubrum* (*Esmarch*) *Molisch* Stamm 4 wurde mittels einer Kompensationsmethode festgestellt. Zwei an einander anschliessende Lichtfelder von verschiedener Farbe wurden auf ein mikroskopisches Präparat von sich bewegenden Spirillen projektiert. Das eine Feld war konstant, während im zweiten die Intensität und die Wellenlänge variieren konnten. Das Bestimmungsprinzip der spektralen Empfindlichkeit der Bakterien beruhte auf der Equivalenzeinstellung beider Felder für verschiedene Wellenlängen. Schreckreaktionen an der Grenze der beiden Felder zeigten die genaue Einstellung an.

Eine mathematische Ableitung wurde aufgestellt. Es wurde gezeigt, dass das Wirkungsspektrum identisch ist mit dem Absorptionsspektrum der Pigmente, welche das in der Phototaxis aktive Licht absorbieren.

Das Wirkungsspektrum zeigt Maxima bei ungefähr 8800, 6900, 5900, 5250, 4900, 4600 und 4000 A.

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